Evidences for Circadian Rhythmicity in the per^0 Mutant of *Drosophila melanogaster*

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per^0 Mutants of *Drosophila melanogaster* which are exposed to light-dark cycles (LD) with different Zeitgeber period (T) have a limited range of entrainment. Entrained flies show a characteristic phase relationship of activity to the LD which depends on the period of the driving cycle as expected by oscillator theory. Both facts are taken as evidence that per^0 possesses endogenous oscillators and that the per gene product is not concerned with central clock structures but rather might be responsible for the mutual coupling between the individual oscillators in a multioscillatory system controlling locomotor activity.

Introduction

The mutant per^0 of *Drosophila melanogaster* which has been isolated by means of chemical mutagenesis [1] is one of the best investigated clock mutants. Its genetics have been traced down to the molecular level [2].

Per^0 is phenotypically arrhythmic with respect to the circadian rhythms of eclosion and locomotor activity [1] as well as with respect to the ultradian rhythm of courtship song of males [3]. It was therefore claimed to be an ideal system for studying the mechanisms of circadian clocks with genetic methods under the assumption that the per gene encodes functions "centrally" concerned with clock structures and that per^0 has lost its circadian system. It is, however, unclear whether the per gene product is a component of the oscillator mechanism itself or affects the clock indirectly when abnormal or missing as a result of the mutation.

The following observations indicate that per^0 might be a mutation in which the basic oscillator is not affected and arrhythmicity is not evoked by interference with its mechanism:

(1) Isolated salivary glands of per^0 larvae show a circadian rhythm in the uptake of a fluorescent dye, 3,3′-dihexyloxacarbocyanine iodide. Compared with the wild-type glands, this rhythm has a lower amplitude and higher desynchrony between the individual cells of a gland [4].

(2) Mathematical analysis of the locomotor activity pattern of per^0 flies reveals ultradian and circadian rhythms with dominant periods ranging from 4 to 22 h in 50–70% of the individuals [5].

(3) The locomotor activity of per^0 is entrainable by light-dark cycles (LD 12:12) [6, 7]. Only under free-run conditions per^0 fails to consistently organize its locomotor behaviour into distinct periods of activity and inactivity.

(4) After release from LD to constant conditions the pattern of locomotion becomes not immediately arrhythmic, but shows some rhythmicity in the individual flies for 1 to 20 days [7].

Since it is important to know whether per^0 is a mutant in which the clock mechanism itself is affected by the mutation, we tried to entrain per^0 flies by LD cycles of different periods. If per^0 still contains a circadian system, the phase relationship of the locomotor activity rhythm to the LD cycle should depend on the period of the LD cycle in a way predicted by oscillator theory. Furthermore the range of entrainment should be limited.

Methods

Locomotor activity was recorded in per^0 mutants and in wildtypes "strain" Berlin (WT_B). Flies were individually monitored using infra-red light beams as described previously [8].

Larvae were reared at 20 °C in LD 12:12 on standard medium in which Isabgol replaced agar [9]. Flies used for recording were less than 5 days old. Males and females were used. Temperature in the recording chamber was 22 ± 0.5 °C. Illumination during the LD was achieved by white Osram L 65 W/25 A

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fluorescence tubes. Light intensity was adjusted to 500 lux. During the dark period a continuous red light of rather weak intensity (7×10^{-8} W/cm^2) was used (red fluorescence tube Philips TL 20 W/25 A 032, with primary red cinemoid filter, Rank Strand).

The activity of the flies was monitored under the following Zeitgeber periods: 19 h (LD 9.5:9.5), 22 h (LD 11:11), 24 h (LD 12:12), 26 h (LD 13:13), 27 h (LD 13.5:13.5). Alternating short and long Zeitgeber periods were used to record activity of individual flies as long as lifetime allowed (Fig. 1).

Results

Wild-type flies were entrained by all Zeitgeber periods used. In per°, however, only about 60% of the flies were entrained by the shortest cycle (T =

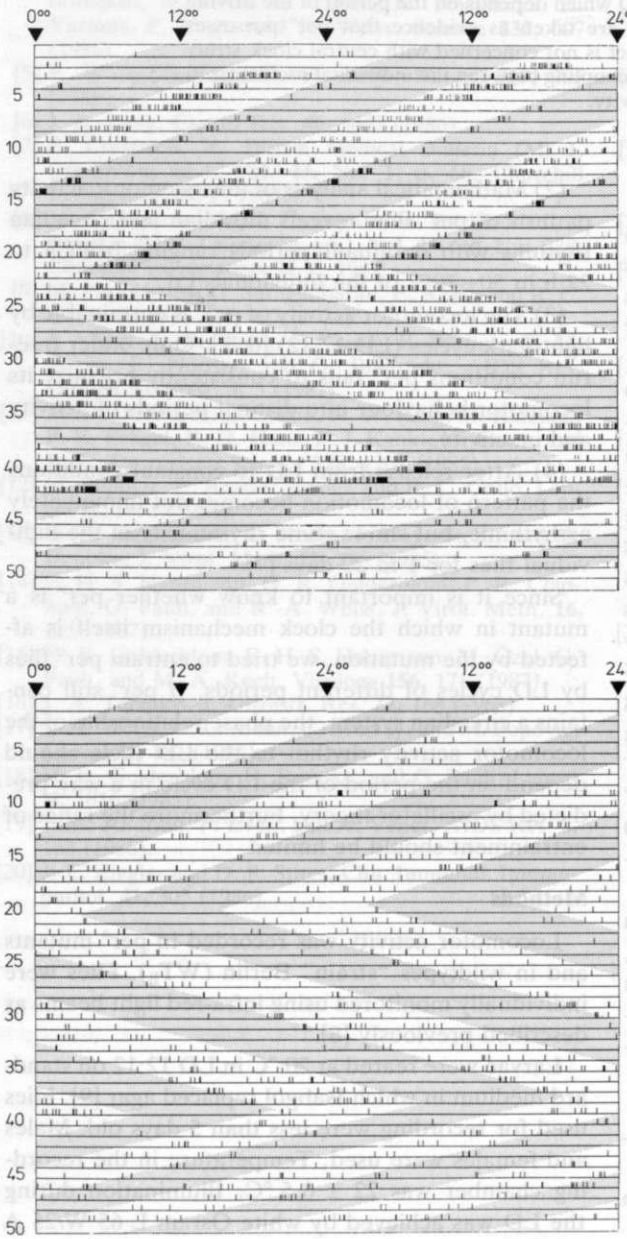

Fig. 1. Examples of actograms (double plotted) of flies entrained to driving cycles with T = 22 h (days 1 to 21 (22 in Fig. 1a)), T = 27 h (days 22 (23) to 39 (40)) and T = 19 h (days 40 (41) to 50). a) Wild-type fly: activity extends in this particular case into darkness for T = 19 h and T = 22 h but not for T = 27 h. b) Per° mutant: activity begins clearly before light-on for T = 27 h and later in the light period for T = 19 h and T = 22 h.

Table I. Percentage of wild-type (WT) and per° flies entrained under LD cycles with different Zeitgeber period (*T*).

T [h]	Percentage of flies entrained WT	*(n)*	per°	*(n)*
19	100.0	(20)	62.5	(24)
22	100.0	(48)	97.4	(38)
24	100.0	(25)	98.0	(35)
26	95.1	(41)	86.0	(43)
27	100.0	(22)	66.7	(30)

19 h) and the longest cycle (T = 27 h), respectively (Table I).

Entrained flies show a characteristic phase relationship of activity to the LD which depends on the period of the driving cycle: activity occurs later in the light period or extends into darkness if short driving cycles are offered and earlier in the light period or even before light-on in long driving cycles. This is found for wild-type as well as for mutant flies (Fig. 1).

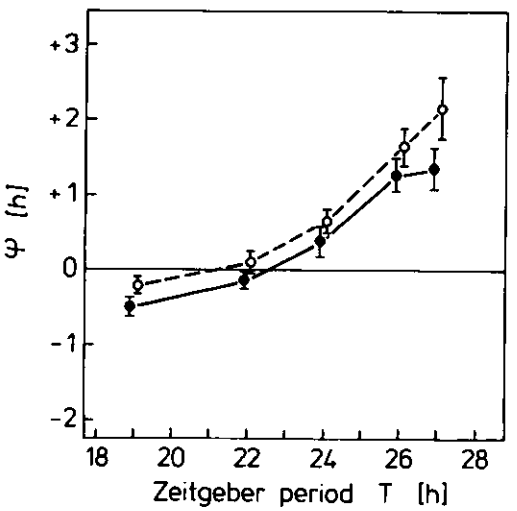

Fig. 2. Phase relationship between begin of activity and onset of light as a function of the period of the driving cycle for wild-type flies (———) and per° mutants (– – –). Positive ψ: Activity begins before onset of light. I, standard error.

In Fig. 2 the mean phase relationship of activity onset to light-on is plotted as a function of the Zeitgeber period. Activity onset is in wild-type and mutant flies before light-on if T = 27 h, 26 h and 24 h and after light-on if T = 19 h and 22 h.

Discussion

The experiments were carried out to test whether the per° mutant has still a functional circadian system controlling locomotor activity. If the circadian system is not functional in the per° mutant, the entrainment by LD cycles must be explained by a direct reaction to light-on and light-off. Activity should therefore begin exactly when light is turned on and end when it is turned off independent of period. In other words, per° must show an unlimited range of entrainment.

Our results show clearly that the range of entrainment of per° is not only limited, but even narrower than that of the wild type. Zeitgeber periods of 19 h and 27 h are already at the limits of entrainment in per° (60% entrained) but not in the wild type (100% entrained). A more extended analysis of the range of entrainment in wild-type flies and per° mutants is under investigation and will be published elsewhere together with further results and an oscillator model to describe our findings.

The second argument against a loss of the circadian system in per° is the fact that the flies do not show a simple light-on and light-off reaction. Activity onset clearly depends on the period of the driving cycle. Like the wild-type flies per° mutants are active before light-on in longer driving cycles and begin activity later in the light period in shorter driving cycles. This is further evidence that per° possesses endogenous oscillators which follow the laws of oscillator theory.

We conclude that, whereas the per gene product is necessary for the normal output of the clock system controlling locomotor activity, it is obviously not concerned with central clock structures. The per gene product might for example be responsible for the mutual coupling between the individual oscillators in a multioscillatory system controlling locomotor activity [10].

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